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EFFECTS OF RICE-ROOT NEMATODE, HIRSCHMANNIELLA ORYZAE (VAN BREDA DE HAAN 1902) LUC AND GOODEY 1963) ON RICE SEEDLINGS.

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1971
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EFFECTS OF RICE-ROOT NEMATODE, *HIRSCHMANNIELLA ORYZAE* (VAN BREDA DE HAAN 1902) LUC AND GOODEY 1963 ON RICE SEEDLINGS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Plant Pathology

by
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B.S., Chulalongkorn University, 1962
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August, 1971

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ABSTRACT

Laboratory observations on feeding of the rice root nematode, Hirschmanniella oryzae (Van Breda de Haan 1902) Luc and Goodey 1963 showed the nematode invaded rice roots in the region of emergence of secondary roots and in the root hair region of the primary root but not at the primary root tip. Feeding was intracellular and caused disruption of cell walls, formation of large cells or cavities and necrosis of roots. Quantitative studies on effects of the nematode were made on seedlings growing in the greenhouse and in a growth chamber. In the greenhouse, the nematode caused significant or highly significant reductions in total root length, root dry weight, shoot length, shoot fresh weight and dry weight of 2-week-old rice seedlings. The stunting effect declined at 4 and 6 weeks after inoculation. In the growth chamber, the reduction in root and shoot measurements was found in 2- and 4-week-old seedlings but not in 6-week-old seedlings.

Bacteria and fungi from nematode suspension water produced no effect on growth of rice seedlings but soil amended with either nematode-free or nematode-infested rice roots caused inhibition of growth. When rice seedlings were grown in soil amended with healthy rice roots and the nematode was added, the stunting effect became more severe.

Infestation of rice seedling roots by H. oryzae caused increases in the level of phenolic compounds and in the activity of polyphenol oxidase, catalase and beta-glucosidase in the roots.

INTRODUCTION

Plant parasitic nematodes are microscopic species belonging to a discrete class of animals. They possess stylets and live mostly in the soil. Interest in these animals has been greatly stimulated where they attack or cause injury to economic plants. Their effects on plants result in damage to plant parts or reduction in plant growth or crop yield. Members of at least 10 genera of nematodes are known parasites on rice plants over the world (30). Hirschmanniella oryzae (Van Breda de Haan 1902) Luc and Goodey 1963 is a parasitic species that invades rice roots and has been considered a major factor in "omo mentek" disease of rice in Indonesia (72). This nematode commonly occurs in paddy fields in Asian countries (32, 55, 65, 66) and causes reductions in growth or yield of rice (38, 41). With reference to the United States, H. oryzae has been known to occur in Texas and Louisiana since 1954 (2). However, no work has been done on the effects of H. oryzae on rice seedlings in the United States.

In this study, the feeding of the nematode on rice roots and the effects of H. oryzae on root tissue were observed under laboratory conditions. Quantitative evidence was obtained as to the effects of various soil treatments on the growth and development of rice seedlings. These treatments included steam sterilized soil, soil amended with both nematode-infested and healthy rice roots and steam sterilized soil plus 800 to 1000 hand-picked specimens of H. oryzae.

The activities of certain enzymes found in root tissue, including those related to the necrosis of root tissue were determined

in seedling roots. Levels of phenolic compounds in rice roots were compared between nematode-infested and nematode-free seedlings.

In summary, this dissertation presents new evidence on the feeding of H. oryzae and on the reactions of the host to this nematode.

REVIEW OF LITERATURE

The rice root nematode now known as Hirschmanniella oryzae (Van Breda de Haan 1902) Luc and Goodey 1963 has been referred to in the past under several different names. As cited by Van der Vecht (73), Soltwedel in 1889 suggested the name Tylenchus oryzae for the nematode found in damaged rice roots. However, he did not describe the nematode. The first satisfactory scientific description of H. oryzae was made by Van Breda de Haan in 1902 under the name Tylenchus oryzae.

In 1931, Imamura (32) put the nematode under the name Tylenchus apapillatus. Goodey (19, 20) named the nematode Anguillulina oryzae. Later the nematode was placed under the binomial Rotylenchus oryzae (15, 16). Thorne (64) established the genus Radopholus and put the nematode in this genus. In 1962, Luc and Goodey (44) changed the name of the nematode to Hirschmannia oryzae. Goodey (22) synonymized Radopholus mucronatus Das, reported in India, to H. oryzae. Because the genus Hirschmannia was preoccupied by a crustacean, Luc and Goodey (45) changed it to Hirschmanniella in 1963.

Bionomics of the Nematode

As cited by Van der Vecht (73), the rice root nematode, H. oryzae, was originally found in Indonesia and was described in 1902 by Van Breda de Haan who called it Tylenchus oryzae. The first report on the occurrence of rice root nematode in Japan was by Imamura (32). He found that the population of this nematode decreased under flooded conditions.

In 1954 H. oryzae was found for the first time in the United States in root-soil samples from rice fields (2). This discovery stimulated a general survey of rice fields for H. oryzae and other nematodes and of studies to determine their agricultural significance. A large number of soil samples were collected from rice fields in Louisiana and Texas (3, 4, 14). From the examinations, eleven species of nematodes belonging to ten genera were found which were considered as parasitic to plants. The other seven species of four genera were suspected plant parasitic nematodes.

Van der Vecht and Bergman (72) made some extensive studies on bionomics of H. oryzae. They reported that the adult nematodes of both sexes enter young roots through the epidermis, at some distance from the root tip and move in both directions. The nematode multiplies in the tissues of the root cortex which become discolored. Oviposition commences a few days after the females have penetrated the roots. The duration of the egg stage does not last longer than a week and probably only about four to five days. The minimum time of development from egg to adult is at least one month and the multiplication factor per generation may be as high as 13.

Thorne (65) collected the nematode in Indonesia, Thailand and the Philippines. From this material he described a most unusual type of development. After the final moult both males and females are about 1 mm long and have very immature gonads. During development they more than double their length and diameter before becoming sexually mature. From his observations Thorne found that various stages of immature and adult nematodes live over in soil between rice crops and during periods of drought. Specimens readily revived when washed from

soil which had been stored dry in a room for eight months. Egg-producing females were not found quiescent in dry soil.

In studies of H. oryzae in the greenhouse on rice plants subjected to a wide range of cultural conditions, Whitlock (75) found an initial rise followed by a general decline in populations. In laboratory studies it was found that the nematode was not extremely susceptible to near freezing temperature, and that it should be able to survive winters throughout most of Louisiana.

It has been generally observed that more nematodes of the species H. oryzae are found in ill-drained paddy fields than in well-drained paddy fields (30, 42, 67).

Yokoo and Naka (81) listed 15 species of the genus Hirschmanniella reported in the world and mentioned that five of them are considered to be parasitic to roots of rice plants. Of these species, only two are found in Saga Prefecture, Japan, and H. oryzae is the dominant species, amounting to 90 percent of mixed populations. They reported that numbers of this nematode isolated from roots is highest in the paddy fields located on the plains (altitude 0-100 meters), and higher in the mountainous districts (above 300 meters) than that in the hilly districts (100-300 meters). By contrast, the numbers of H. oryzae isolated from the soils around rice roots increased with increase in altitude.

Yokoo and Su (78), in studies of changes of nematode populations in paddy fields, found that the numbers of H. oryzae in the soil after removal of water, decreased rapidly, amounting to one-third compared with that in the soil before removal of the irrigation water. This species was distributed in the subsurface (10-20 cm depth) soil layer

most numerously; amounting to 60 percent of the population before the removal of water. Distribution was extensive in 0-20 cm depth soil layer after the removal of water.

Ishikawa, cited by Ichinohe (31), reported that applications of nitrogenous fertilizers increased nematode populations, as well as such plant characters as culm height, number of tillers, root-browning, and rotting of roots, while application of either potassium fertilizers or compost depressed the nematode population level throughout the season. Tomonaga and Kurokawa (67) found a decrease in numbers of parasitic nematodes and an increase in grain yield when either calcium silicate or compost was applied to paddy fields.

When certain soil improving materials, consisting of several chemicals, was added to the rice paddy field, it was found that the numbers of Hirschmanniella spp. decreased up to 15 percent compared with that of unamended fields, while the number of free-living and carnivorous nematodes increased. The application of soil-improving materials resulted in increase of grain yield from 3.5 to 5.0 percent (79, 80).

Damage caused by the nematode to the rice plant

As cited by Van der Vecht (73), Van Breda de Haan reported in 1902 the occurrence of rice root nematode in Indonesia and he considered it to be the causal agent of a rice disease called "omo mentek". In 1912 Van der Elst advanced the conclusion that mentek is a physiological disease and that the rice root nematode is of no practical importance in this connection. Kuilman, who was Van der Elst's successor, also took the same view and the nematode was almost completely forgotten for approximately 40 years.

Van der Vecht and Bergman (72) studied extensively the rice root nematode and its effects on the host. They found that the nematode multiplies in the tissues of the root cortex; the cortex becomes discolored and growth of the plant is retarded. The effects are complex since they depend on the number of nematodes, age of the attacked plant, the soil and weather conditions and the variety of rice. They believed that there exists a relation between the nematode infestation and mentek disease of rice and that the mentek disease is likely to break out under conditions which do not enable the plant to recover sufficiently to counteract the serious injury resulting from nematode attack.

Timm and Ameen (66) reported the occurrence of symptoms of "mentek" disease of rice caused by H. oryzae for the first time in Pakistan in 1960.

It has been commonly observed that the nematode causes a retardation of plant growth as evidenced by decreases in plant height, and fresh and dry weights of plants (38, 41, 67, 72).

Kawashima and Fujinuma (41) reported from laboratory tests that the nematode caused retardation of growth of rice seedlings. They found rice roots became brownish in color and that this discoloration of roots increased in accordance with the amount of inoculum ($r = 0.991$). They interpreted their observations to mean that the nematode caused changes in physiological functions of rice roots, resulting in a staining with iron oxide. They found that oxidative activity of rice roots was less in nematode-infested than in nematode-free roots.

However, it is my observation that brown discoloration of rice roots seems to occur commonly in rice and is not confined to rice infested with nematodes. Takijima (62) reported that the rusty-brown bands on rice roots were caused by accumulation of ferric hydroxide. Sturgis (60) found that the incrustation formed on and around older rice roots was composed of iron and manganese oxides.

Kawashima (39) reported that the invasion and parasitism of roots by the nematode starts several days after rice transplantation. The invasion is rapid at high temperatures; the optimum temperature is 25-30 C and the death of cells is seen to occur after invasion. Because of the accumulation of substances stained with hematoxylin near the head of the nematode, he believed that substances secreted by the nematode caused the death of cells.

Kawashima (40) studied injury of rice plants caused by the rice root nematode in relation to biochemically reduced paddy soil in pots. Examinations indicated that, under reduced soil conditions, the nematode decreases the number of tillers more definitely than it decreases the plant height, and that these decreases became greater as the soil reduction proceeded. The yield decrease caused by the reduction of soil was much higher in the nematode-inoculated pots than in the uninoculated ones.

Rao and Panda (55) have reported on the extensive studies made in India on rice soil nematodes supported by funds supplied under U. S. Public Law 480. The results showed that infestation of seedlings by Hirschmanniella mucronata (Das) (Hirschmanniella oryzae) produced promotion effects, or an increase of tillering, root length and root weight, but not of grain yield. With an increase in the

level of inoculum, there was an increase in tiller production, particularly in plants which were inoculated at either 1 or 40 days of age. Root length and root fresh weight of plants inoculated at 1 day age increased with increase in the level of inoculum. When 1-day old rice seedlings were inoculated with nematodes at levels of 5 and 10 thousand per plant, grain yield decreased 50.6 and 70.0 percent. In the inoculations made on 40-day old seedlings, yield reductions were 43.0 and 65.5 percent, respectively, at the level of 5 and 10 thousand nematodes per plant.

Kawashima (37) made an estimation of the susceptibility of 17 rice varieties to the nematode, based on the number of nematodes extracted from the roots. He found no significant differences among varieties. Van der Vecht and Bergman (72), working on two varieties of rice, found that one variety was 4 to 5 times more heavily infested than the other.

Kawashima (38) reported a successful controlling effect of D-D mixture (1,3-dichloropropene-1,2-dichloropropane mixture) against H. oryzae in a well-drained paddy field. Increase in rice yield in the second year after treatment was 27 percent compared with that of nontreated plots.

Taylor et al. (63) conducted field experiments for nematode control in Thailand. In one of these experiments the yield of rice from seedlings in seedbeds treated with nematocides and transplanted to nematocide-treated paddies was increased from 24.3 to 30.6 percent in various treatments as compared with the controls. In another experiment, the growth of transplanted rice was increased 52.6 percent by treatment of only the paddy, 56.2 percent by treatment of only the

seedbed, and 93.1 percent by treatment of both the seedbed and paddy. In this location, the only nematode infecting rice in the paddy was a species of Hirschmanniella.

Yamsonrat (77) reported the results of a pot experiment in which the rice root nematodes (Hirschmanniella spp.) caused reductions in straw weight, root weight and grain yield. The decrease in grain yield was 32 percent.

Whitlock (75) conducted a pot experiment on H. oryzae in the greenhouse and reported that the nematode caused no damage to germinating seedlings and young rice plants.

In 1969, a field experiment was conducted in California with 2 nematocides for the control of Hirschmanniella belli Sher 1968. The results showed that there was no significant difference in rice yield between treated and nontreated plots (49).

MATERIALS AND METHODS

Nematode-Host Reaction Studies

Rice plants of the variety Saturn were grown in a mixed soil composed of one part builder's sand and three parts steam-sterilized field soil. This mixture was put in a plastic bag to the amount of one and a half kilograms and the bag was then placed in a 6-inch clay pot. For the nematode-infested treatment, 800 hand-picked specimens of Hirschmanniella oryzae (Van Breda de Haan 1902) Luc & Goodey 1963 were mixed in the surface soil in the pot one day before planting. Rice seeds were soaked in water over night and then kept in moisted petri dishes for four days. Seedlings were selected for uniform root and shoot lengths. Ten seedlings were planted per pot at a 1 cm depth. Three days later they were thinned to six seedlings per pot. In a greenhouse experiment, rice seedlings were planted on February 24, 1970. In a growth chamber experiment, the same number of rice seedlings were planted on July 31, 1970. The chamber was set for a 14 hr day, a 10 hr night and for a light intensity of 1200 foot-candles. The temperature was adjusted to 26 C during the day and 20 C during the night. The soil in each pot was kept flooded at the same depth with tap water.

In these experiments the average number of nematodes per seedling was 133, and the number per kilogram of soil was 533, or about 0.5 nematode per gram of soil.

Observations were made at 2, 4, and 6 weeks after planting, and these were regarded as the ages of seedlings. Plastic bags, each

containing 6 seedlings were removed from the pots and brought back to the laboratory. Plants in the bags were extracted by washing the soil away from the roots slowly so as to avoid damage to the root system. At the end of each growth period a count of the nematodes in the soil was made on all of the treatments.

The roots of the six seedlings from each container were separated carefully from each other then cleaned in running water and placed separately in beakers of water. The seedlings, then, were ready for measurements which included length of shoots, shoot fresh weight and dry weight, root length, root number and dry weight of roots. The water in each beaker was kept for a count of the nematodes moving out of the roots into the water.

Shoots were cut off to measure length and fresh weight and were put into an oven for dry weight determinations. Measurement of lengths and numbers of primary roots was done in a glass tray with a plastic ruler placed beneath the tray. The number of secondary roots were counted under a binocular microscope by spreading the roots out on a 5 x 7 inch glass plate. Five to ten percent of the total secondary roots were sampled for measurements of length and diameter. The average diameters of primary and secondary roots were measured with a calibrated ocular micrometer under the microscope. After these measurements were completed the roots were stained with acid fuchsin by the method of Goodey (21).

Counts of the numbers of nematodes were done under a binocular microscope. The total number of nematodes inside the roots was those nematodes found in stained roots plus those found in the water in which the roots were soaked. Roots were left in water for a few days

to remove the stain and lactophenol, then dried in an oven to prepare for determination of dry weight. Data of all measurements were analyzed by the t-test (43).

Nematode-Soil Amendments

The composition of mixed soil was the same as in the previous experiments. Three kilograms of this mixed soil was put in each of fourteen 7-inch crocks. Then two crocks were used for each treatment. In the first treatment, 1000 hand-picked specimens of H. oryzae were mixed in the surface soil of each crock. In the second treatment, soil in each crock was mixed thoroughly with 33 grams of rice roots from 10-week old seedlings planted in steam sterilized soil; then 1000 specimens of the nematode were added per pot. In the third treatment, soil in the crock was mixed with 33 grams of rice roots from 10-week old seedlings planted in field soil. In the fourth treatment, soil in each of the two crocks was mixed with 33 grams of rice roots as in the second treatment, but without the nematode added. The fifth treatment was made by adding to each crock 100 ml nematode-free wash water collected from washing 4000 specimens of H. oryzae. In the sixth treatment, a culture of microorganisms was added to the surface soil of the crock. This was prepared by streaking three potato dextrose agar plates with water from the nematode suspension used in treatment five and incubating the plates at room temperature for three days. The organisms - fungi and bacteria in the resulting cultures - were washed from the plates with distilled water and made up in suspension to a volume of 100 ml. Fifty ml of this suspension was added to the soil in each crock. In treatment seven, the crocks contained only steam sterilized field soil and served as controls.

To each pot, eight 4-day old rice seedlings were planted on October 23, 1970, one day after soil preparation. Then the seedlings were thinned to 5 per pot. The plants in each pot were kept flooded at the same depth with tap water. Observations were made on November 21, 1970. In this experiment, an average of 200 nematodes were used for each seedling. The soil was inoculated with 333 nematodes per kilogram of soil, or 0.3 nematode per gram of soil. Individual comparisons in this experiment were made by Duncan's multiple range test (43).

Preparation of rice seedlings for determination of phenolic compounds and enzymatic activities

Rice seeds, variety Saturn, were surface sterilized with 10 percent Clorox (0.525 percent Sodium Hypochlorite) for 1 hr, rinsed several times with sterilized distilled water and soaked overnight. The seeds were kept moist in a petri dish for three days. One percent water agar was prepared and 30 ml amounts were put into 250-ml beakers. The beakers containing water agar were sterilized in an autoclave at 15 psi for 15 min. Three sprouted seeds were embedded per beaker. On the next day, 150 hand-picked specimens of H. oryzae were introduced into the agar (inoculated) around the three rice seedlings and the cultures were maintained at 25 C.

Determination of phenolic compounds in rice roots and nematodes

Samples of either nematode-infested or nematode-free roots were ground in a small mortar and extracted for phenolic compounds with boiling 95 percent ethanol. The homogenates were strained through four layers of cheesecloth. The residues were then reextracted once more with ehtanol and the combined filtrates centrifuged at 1500 g

for 15 min. Final volumes of the supernatants were adjusted to 10 ml per gram fresh weight of roots. Then duplicate 1 ml aliquots were used. Phenolic compounds were extracted in the same manner from Hirschmanniella oryzae, after one thousand nematodes of this species had been washed 3 times with distilled water and then ground with glass wool in a mortar.

Total phenols were determined by the method of Swain and Hillis (61), except for minor variations in the amount of tissue extracted and reagents used. One ml of the ethanol extract was pipetted into a 10-ml test tube containing 1 ml of distilled water, one and a half ml of Folin-Denis reagent (17) were added and the tubes were thoroughly shaken. After holding the sample 2 to 3 min, 5 ml of saturated sodium carbonate solution was added and the contents were mixed well by shaking the tube. The blue color was allowed to develop for 1 hr then the mixture was centrifuged at 5000 g for 15 min. The supernatant was used for determining absorptivity at 660 mμ (35) in a Bausch & Lomb Spectronic 20 spectrophotometer. A mixture of 2 ml distilled water, 1.5 ml Folin-Denis reagent and 5 ml saturated sodium carbonate solution served as a control. Chlorogenic acid was used as a standard for calculating total phenol. The amount of phenolic compounds was expressed, based on chlorogenic acid, as milligrams per gram fresh weight of roots. With respect to nematodes, the amount of phenolic compounds was expressed as micrograms per 1000 nematodes.

Determination of enzymatic activity in rice roots and nematodes

A) Polyphenol oxidase acticity:

Enzyme extract was prepared by grinding either nematode-infested or nematode-free roots in 0.2 M sodium phosphate buffer at pH 7.0 for

2 min at high speed in a Sorvall Omni-mixer. The resulting homogenate was strained through four layers of cheesecloth and the filtrate was centrifuges at 1500 g for 15 min at 4 C. The supernatant fluid was added with the same buffer to the final volume of 1.0 ml per 0.07 g root fresh weight and used for the enzyme assays.

Polyphenol oxidase activity was determined by the method of Maxwell and Bateman (46). The reaction mixtures contained 1.0 ml enzyme extract, 2.0 ml distilled water, 1.0 ml 0.2 M sodium phosphate buffer at pH 7.0, and 1.0 ml of (2 mg/ml) catechol. The same mixture was used in the control except that enzyme extract was replaced by boiled enzyme extract. The enzyme activity was expressed as the change in absorbance at 495 mμ versus time (hr).

B) Catalase activity:

The catalase enzyme activity of rice roots was determined by the method of Rodriguez-Kabana and Truelove (56), modified by Pitts (52) for polarography apparatus (YSI Oxygen Monitor Model 55). Fifty-milligram samples from either nematode-infested or nematode-free rice roots were ground in a small mortar. To the reaction vessel was added the triturated roots and 3.9 ml of deionized water. When a steady trace was recorded with a YSI model 80 recorder, 0.1 ml of a 1.0 percent H_2O_2 solution was injected into the suspension. The enzyme activity of 300 nematodes was determined on nematode samples prepared by washing the nematodes 3 times with distilled water and grinding them in a mortar with glass wool. The method of determination was the same as with the roots. The enzyme activity was expressed as the slope of the curve = tan σ.

C) Beta-glucosidase activity:

Beta-glucosidase enzyme extracts of nematode-infested and nematode-free rice roots were prepared by the method used for polyphenol oxidase, except 0.2 M acetic acid - sodium acetate buffer at pH 5 was used instead of sodium phosphate buffer at pH 7. The suspension was autolysed for 1 hr at 37 C before centrifugation. The final volume was adjusted to 1.0 ml per 0.04 g root fresh weight. Assay of the enzyme activity was made immediately after preparation of the enzyme extract.

For preparation of the nematode homogenate, 1000 hand-picked nematodes were washed 3 times with distilled water. After the third washing and draining, 2 ml of distilled water was added and centrifuged to move nematodes to the bottom. One ml of this distilled water was kept as a control; the other 1 ml with nematodes in it was incubated for 12 hr at 25 C. Then this 1 ml of water was tested for beta-glucosidase activity excreted or secreted by the nematode. A homogenate of the nematode in citrate-phosphate buffer, pH 4.5, was prepared by supersonic sound vibration, after fine glass beads were added to the treatment chamber. The homogenate was cnetrifuged at 5000 g for 10 min and the clear supernatant was used to determine the enzyme activity.

Beta-glucosidase activity in nematode-infested and nematode-free rice roots was determined by the method of Gelman (18). The incubation mixture contained 2.0 ml of 0.2 M Na_2HPO_4 - 0.1 M citric acid buffer at pH 4.5, 1.0 ml enzyme preparation and 0.25 ml of 0.1 M p-nitrophenyl-beta-D-glucopyranoside. The volume was made up to 4.0 ml with distilled water and after incubation for 1 hr at 37 C, 4.0 ml of 0.4 M

glycine-NaOH buffer, pH 10.8, was added to terminate the reaction and to develop the color of the liberated p-nitrophenol. The resultant color intensity was read at 400 mμ (76) in a Bausch & Lomb Spectronic 20 spectrophotometer. From a standard curve the amounts of p-nitrophenol released were determined.

Beta-glucosidase activity in nematodes was determined by the method of Wilski and Giebel (76). The reaction mixture contained 1.0 ml nematode homogenate in buffer at pH 4.5, 1.0 ml distilled water and 0.5 ml of 0.1 M p-nitrophenyl-beta-D-glucopyranoside. Where nematode wash water served as a control or for determination of excreted or secreted enzyme, 1.0 ml samples were used. One ml citrate phosphate buffer at pH 4.5 and 0.5 ml of 0.1 M p-nitrophenyl-beta-D-glucopyranoside were added and the mixtures were incubated at 32 C for 24 hr, after which 3.0 ml of 1.0 M sodium carbonate was added to each series of these reaction mixtures. The intensity of released p-nitrophenol was read as in rice roots.

RESULTS

General Observations on the Feeding of H. oryzae on rice roots

Feeding of Hirschmanniella oryzae on rice roots was observed in the laboratory with the use of special chambers constructed by Hollis et al. (25). The method for the culture and inoculation of rice seedlings was the same as that used for determination of phenolic compounds and enzymatic activities, except that the seedlings were grown in chambers instead of beakers. The chambers contained 1% water agar in a layer 1 cm thick. Observations were made by inversion of the chamber on the microscope stage.

Within 24 hr after inoculation, several nematodes were found moving along the primary seminal root and occasionally stopping and pressing the root with their anterior ends. The nematodes invaded the piliferous region of the root and the region in which secondary roots emerged; they were never found feeding on the root tip or on secondary roots.

Within 48 hr after inoculation, brown lesions occurred on roots at a distance of 1 to 2 cm from the seeds (root length at this stage was about 4 cm). Examination of brown lesions under a microscope revealed brown patches on the surface of the root. Usually 1 lesion developed on a root, but 2 lesions were sometimes found 3 to 5 mm apart. The lesions usually occurred first on the under side of a root (the side that faces the bottom of the chamber). The brown necrotic lesions were sometimes only small spots but mostly they were elongated

up to 1 cm in length. The length of brown lesions increased with time after inoculation.

Three to four days after inoculation of the rice seedling chambers with H. oryzae, secondary roots were found to emerge from the primary root. In some cases, secondary roots in the necrotic area turned brown and never emerged from the primary root. Sometimes necrosis occurred on one side of the root and secondary roots were produced on the other side.

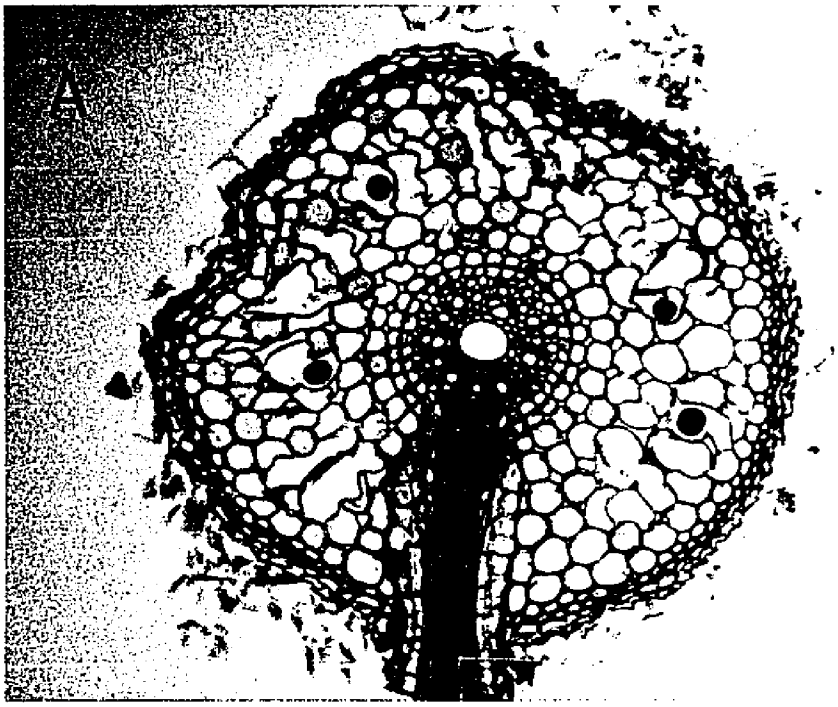
Seven days after inoculation the seedlings were pulled out from the agar and the roots were fixed in formalin-acetic acid-alcohol solution (FAA). Root tissues were embedded by the paraffin method and sections were cut 16 microns thick with a rotary microtome and stained with safranin and fast green.

Stained transverse sections of roots cut in the vicinity of infection sites, showed that H. oryzae took up feeding positions at random in parenchyma cells of the cortex. The feeding was always intracellular (Fig. 1-A). On penetration, the nematode made a tunnel which was almost perpendicular to the root surface (Fig. 1-B). The depth of penetration was variable. After penetration, the nematode usually became orientated parallel to the long axis of the root and moved either up or down; nematode movement occurred in both directions. In some instances, the nematode moved transversely across the cortex and damaged 5 or 6 parenchyma cells (Fig. 2-A). Single nematodes feeding in the root did not always disrupt adjacent cells (Fig. 1-A); but when several nematodes were associated with an infection, a cavity was always formed which included them (Fig. 2-B). In some cases a rounded large cell was developed (Fig. 3).

Figure 1. Transverse sections of rice roots showing the rice root nematode (Hirschmanniella oryzae) in cortical cells.

A) Four cortical cells, each containing a transverse segment of a single nematode.

B) Tunnel made by a nematode entering the cortex.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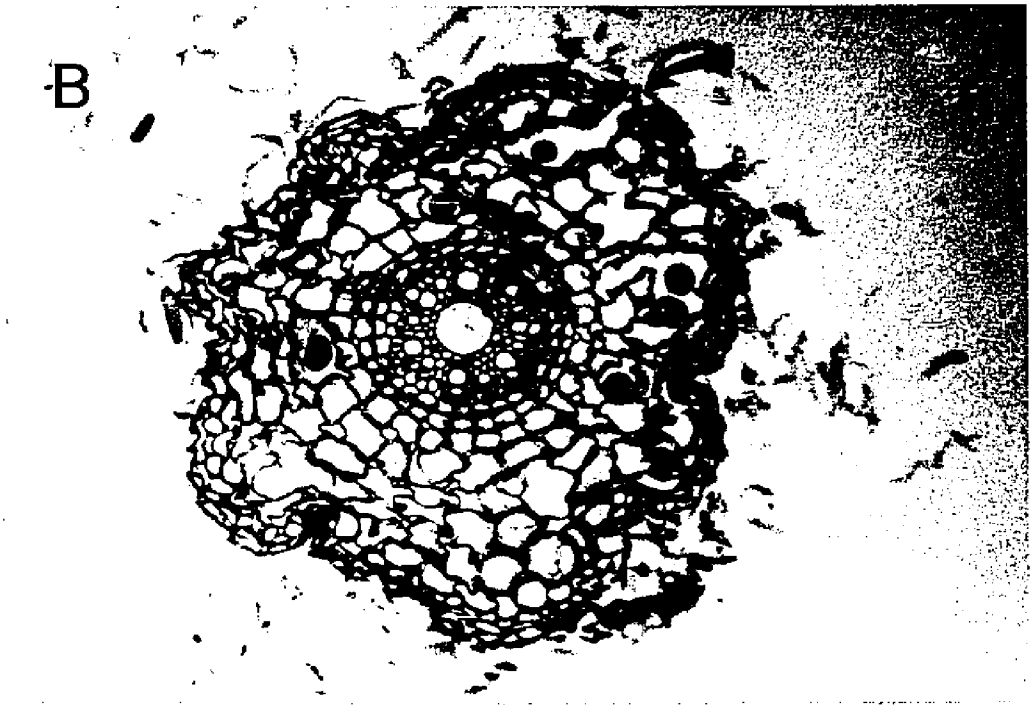
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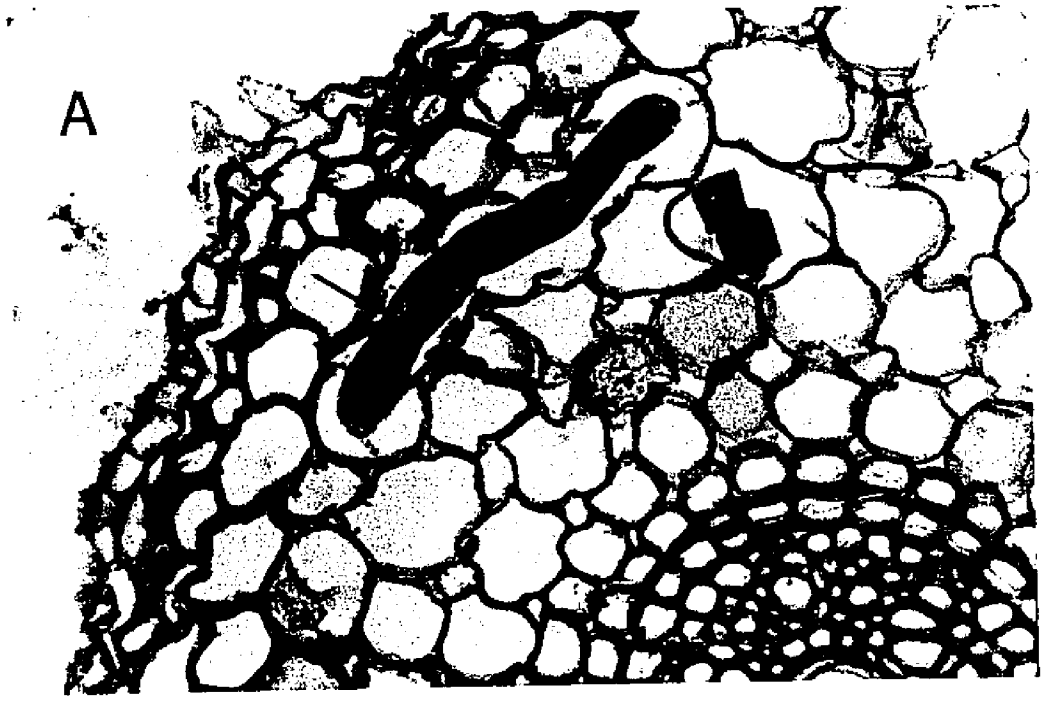
Figure 2. Transverse sections of rice roots showing the rice root nematode (Hirschmanniella oryzae) in cortical cells.

A) One nematode occupying several cortical cells.

B) Cavity in cortex occupied by three nematodes seven days after inoculation of seedlings.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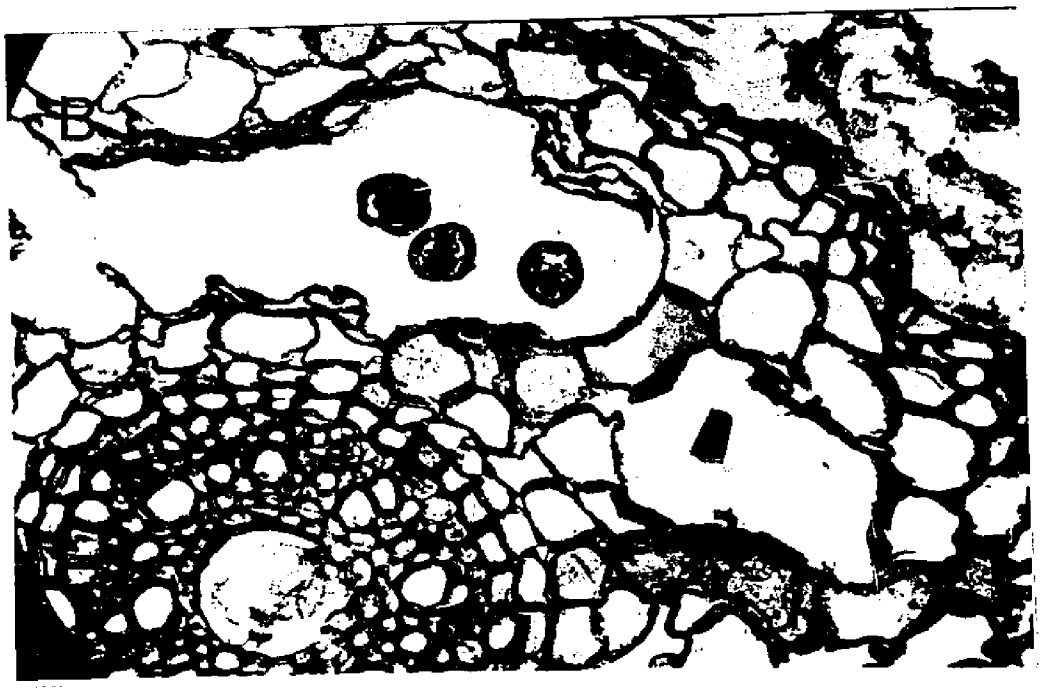
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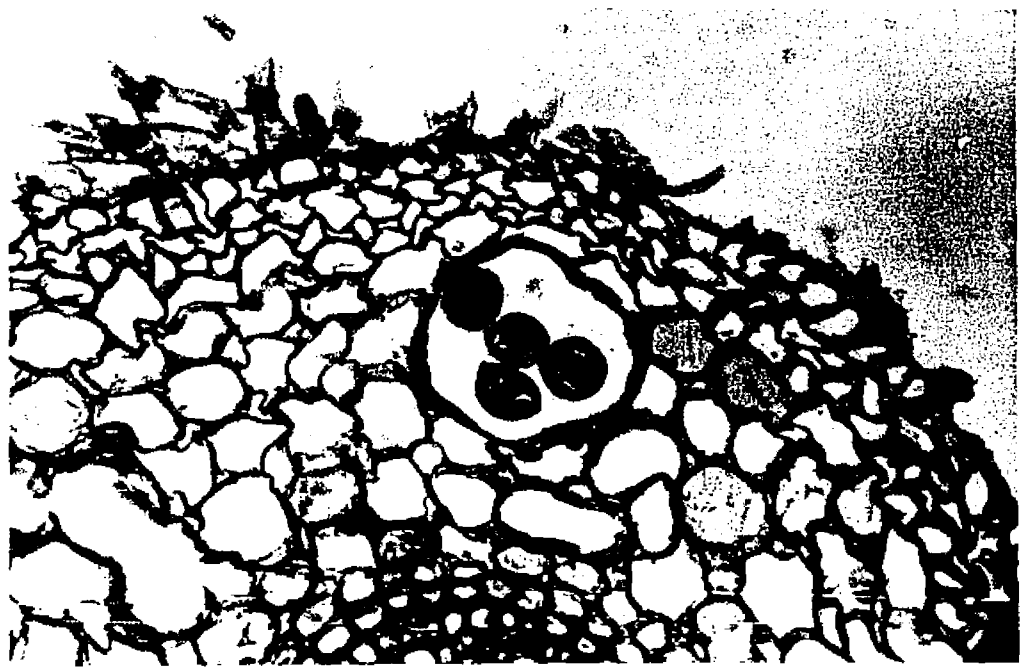

Figure 3. Transverse section of root cortex showing a rounded large cell containing four specimens of the rice root nematode (Hirschmanniella oryzae) in transverse section seven days after inoculation of seedlings.

Nematode-Host Reaction Studies

Effect of *Hirschmanniella oryzae* on rice seedlings in the greenhouse:

Roots of rice seedlings grown in steam-sterilized field soil were healthy and white. In infested plants, roots showed brown lesions at a distance of a few centimeters from the culms. No knots or other abnormalities were seen on the infected roots. The primary or solitary seminal root was attacked first; then adventitious roots were attacked. The brown lesion expanded on an individual root and at last the whole root rotted. Young, new-born roots which had not been attacked by the nematode were white.

Tables 1, 2 and 3 show means of six measurements of roots and shoots. Table 4 shows the statistical significance of the results presented in Tables 1-3. When the data from two-week-old seedlings were analyzed statistically, there were some significant differences between the control and the treatment with nematodes, except for the observation on the number of roots in which the reduction was not statistically significant. The nematode cuased highly significant reductions in root dry weight, shoot fresh weight and dry weight. Significant reductions were found in total root length and length of shoot.

Four-week-old seedlings data showed significant reductions in root dry weight and shoot dry weight. For measurements of total root length, total number of roots, length of shoot and shoot fresh weight, the data showed reductions but they were not statistically significant.

Table 1. Mean[a] of length, number, dry weight of roots and length, fresh weight and dry weight of shoots (two-week old seedlings in greenhouse).

Treatment		Total root length cm	Total no. of roots	Root dry weight g	Shoot length cm	Shoot fresh weight g	Shoot dry weight g
Nematode-infested	Mean	94.14	137.00	0.0018	28.25	0.1293	0.0211
	Standard deviation	31.34	36.57	0.0006	4.83	0.0173	0.0027
	Standard error	12.79	14.93	0.0002	1.97	0.0071	0.0011
Nematode-free	Mean	157.21	226.33	0.0036	33.33	0.1728	0.0271
	Standard deviation	57.48	91.93	0.0007	1.82	0.0265	0.0030
	Standard error	23.46	37.52	0.0003	0.74	0.0108	0.0012

[a] Average of six rice seedlings.

Table 2. Mean[a] of length, number, and dry weight of roots and shoot length, fresh weight and dry weight (four-week old seedlings in greenhouse).

Treatment		Total root length cm	Total no. of roots	Root dry weight g	Shoot length cm	Shoot fresh weight g	Shoot dry weight g
Nematode-infested	Mean	942.34	1252.00	0.0189	46.05	0.8178	0.1227
	Standard deviation	231.07	231.69	0.0046	3.43	0.2123	0.0304
	Standard error	94.31	94.57	0.0019	1.40	0.0867	0.0124
Nematode-free	Mean	1317.99	1587.17	0.0258	49.73	1.0439	0.1567
	Standard deviation	397.83	411.07	0.0051	2.58	0.1460	0.0189
	Standard error	162.38	167.78	0.0021	1.05	0.0596	0.0077

[a] Average of six rice seedlings.

Table 3. Mean[a] of length, number, and dry weight of roots and shoot length, fresh weight and dry weight (six-week old seedlings in greenhouse).

Treatment		Total root length cm	Total no. of roots	Root dry weight g	Shoot length cm	Shoot fresh weight g	Shoot dry weight g
Nematode-infested	Mean	1478.17	1490.33	0.0649	56.98	1.6643	0.3284
	Standard deviation	496.33	515.51	0.0146	2.88	0.2176	0.0258
	Standard error	202.58	210.41	0.0060	1.18	0.0888	0.0105
Nematode-free	Mean	2523.94	2372.83	0.1949	58.85	1.8753	0.3373
	Standard deviation	355.22	330.94	0.0330	1.13	0.1864	0.0468
	Standard error	144.99	135.08	0.0135	0.46	0.0761	0.0191

[a] Average of six rice seedlings.

Table 4. Effect of Hirschmanniella oryzae (Van Breda de Haan, 1902) Luc & Goodey, 1963 on rice seedlings growing in greenhouse (statistical significance of the data presented in Tables 1-3).

Seedling age (weeks)	Measurement	Treatment mean difference (nematode-free) - (nematode-infested)	Standard error of mean difference	t value
2	Total root length	63.07	26.72	2.360*
	Total root number	89.33	40.38	2.212
	Root dry weight	0.0018	0.0004	4.500**
	Shoot length	5.08	2.10	2.419*
	Shoot fresh weight	0.0445	0.0129	3.450**
	Shoot dry weight	0.0060	0.0013	4.615**
4	Total root length	375.65	187.78	2.000
	Total root number	335.17	192.60	1.740
	Root dry weight	0.0069	0.0028	2.464*
	Shoot length	3.68	1.75	2.103
	Shoot fresh weight	0.2261	0.1052	2.149
	Shoot dry weight	0.0340	0.0146	2.329*
6	Total root length	1045.77	249.12	4.197**
	Total root number	882.50	250.04	3.529**
	Root dry weight	0.1300	0.0467	2.784*
	Shoot length	1.8800	1.2700	1.480
	Shoot fresh weight	0.2110	0.1169	1.805
	Shoot dry weight	0.0089	0.0218	0.4083

* Significant (at 5% level of probability).

** Highly significant (at 1% level of probability).

In six-week-old seedlings, the nematode caused highly significant reductions in total root length and total root number, and a significant reduction in root dry weight. Reductions of shoot length, shoot fresh weight and dry weight were not statistically significant.

Means of diameters of roots at three different ages and their statistical analyses are shown in Table 5. The nematode caused reductions in diameters of both primary and secondary roots of all ages but these reductions were not statistically significant except for the secondary roots in six-week-old seedlings.

Root-shoot ratios of rice seedlings are shown in Fig. 4. The ratios were slightly increased with increase in age of plant and were highest in six-week-old seedlings.

Figure 5 shows the average number of the nematodes per seedling and calculated number per gram dry weight of roots. The number of nematodes per seedling increased from 2.8 in two-week-old seedlings to 18.3 and 32.8 in four- and six-week-old seedlings, respectively. By contrast, the calculated number of nematodes per gram dry weight of roots decreased from 1629.7 in two-week-old seedlings to 1024.7 in four-week-old and 545.0 in six-week-old seedlings.

Effects of Hirschmanniella oryzae on rice seedlings in a growth chamber:

When rice seedlings were grown in a growth chamber under controlled conditions, the seedlings produced more roots and the root-shoot ratio was higher than in the greenhouse (Fig. 4). The root-shoot ratio was lowest in two-week-old seedlings, intermediate in six-week-old seedlings, and highest in four-week-old seedlings.

Table 5. Diameters[a] of primary and secondary roots of rice seedlings grown in the greenhouse in nematode-free and nematode-infested soil.

Seedling age (weeks)	Primary roots (microns)			Secondary roots (microns)		
	Nematode-infested	Nematode-free	t value	Nematoed-infested	Nematode-free	t value
2	480.99	511.05	0.929	89.56	95.49	1.699
4	629.22	648.71	0.692	87.91	90.32	1.840
6	715.71	736.69	0.872	86.40	93.71	3.973**

[a] Mean diameters of roots from 6 seedlings.

** Highly significant (at 1% level of probability).

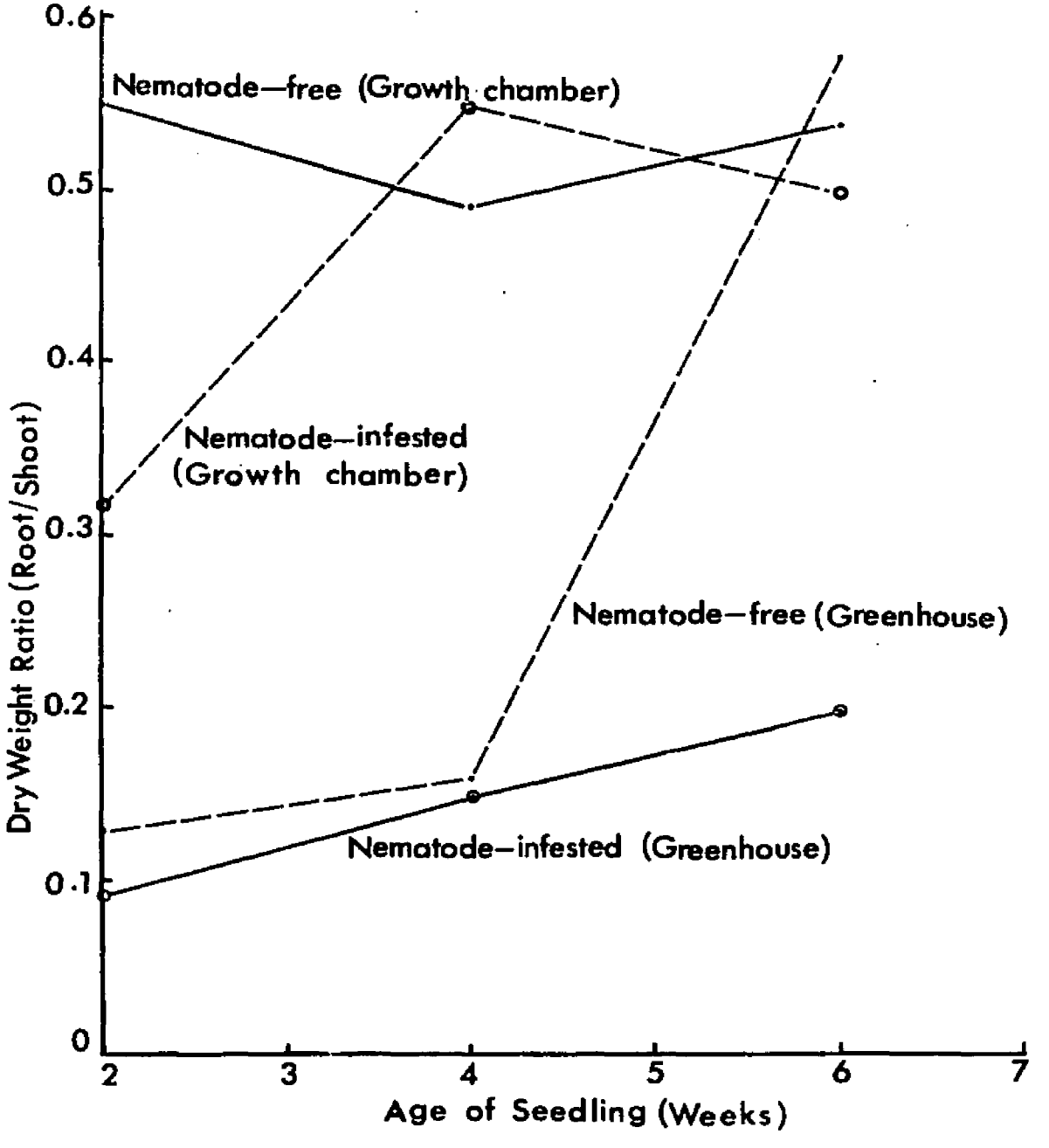

Figure 4. Root-shoot ratios of rice seedlings growing in the greenhouse and in a growth chamber.

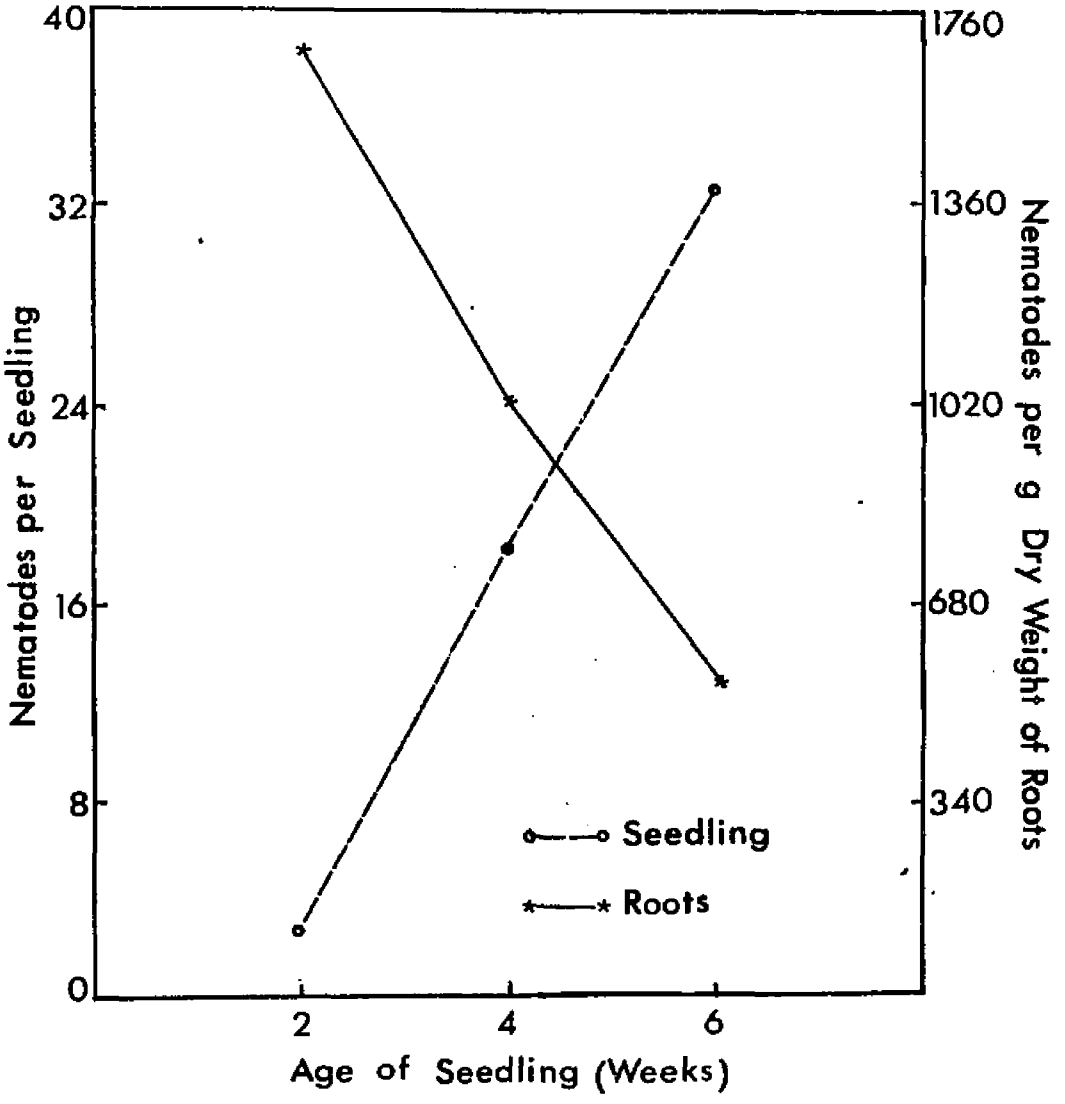

Figure 5. Number of nematodes per rice seedling and calculated number of nematodes per gram dry weight of roots.

Tables 6, 7 and 8 show means obtained from measurements made on rice seedlings at 2, 4 and 6 weeks of age, respectively. Table 9 shows the statistical significance of the results presented in Tables 6, 7 and 8. When the data from two-week-old seedlings were analyzed statistically, there was a significant difference in all cases between the control treatment and the treatment with nematodes. H. oryzae caused significant reductions in total length of roots, total root number, length of shoot, shoot fresh weight and dry weight, and it caused a highly significant reduction in root dry weight.

Four-week-old seedling data showed a significant difference between the control treatment and the treatment with the nematode in all measurements except that of the root dry weight. The nematode caused highly significant reductions in total root length and length of shoot. Significant reductions were found in total root number, shoot fresh weight and dry weight.

The data of six-week-old seedlings showed no significant differences between the control and the nematode-infested treatments.

Table 10 shows the mean differences in diameters of roots and their statistical significance. The nematode caused significant or highly significant reductions in diameters of primary and secondary roots at all ages of seedlings, with the exception of the diameters of the primary roots in four-week-old seedlings.

Nematode-Soil Amendments

The results in Table 11 indicate that neither the water from the nematode suspension nor the culture of bacteria and fungi from the water of the nematode suspension caused a statistically significant reduction in total root length; however in other treatments, a reduction in root length was found. Noninfested rice roots when added

Table 6. Mean[a] of length, number, and dry weight of roots and shoot length, fresh weight and dry weight (two-week old seedlings in growth chamber)

Treatment		Total root length cm	Total no. of roots	Root dry weight g	Shoot length cm	Shoot fresh weight g	Shoot dry weight g
Nematode-infested	Mean	1894.08	1183.08	0.0351	33.05	0.5288	0.1113
	Standard deviation	1101.02	474.94	0.0159	2.00	0.0937	0.0240
	Standard error	317.84	137.10	0.0046	0.58	0.0270	0.0069
Nematode-free	Mean	2752.80	1622.17	0.0808	34.98	0.6598	0.1469
	Standard deviation	659.49	326.05	0.0297	2.49	0.1624	0.0401
	Standard error	190.38	94.12	0.0086	0.72	0.0469	0.0116

[a] Average of 12 rice seedlings.

Table 7. Mean[a] of length, number, and dry weight of roots and shoot length, fresh weight and dry weight (four-week old seedlings in growth chamber).

Treatment		Total root length cm	Total no. of roots	Root dry weight g	Shoot lenght cm	Shoot fresh weight g	Shoot dry weight g
Nematode-infested	Mean	2874.78	1873.00	0.1184	37.29	0.7886	0.2153
	Standard deviation	742.37	440.35	0.0229	1.63	0.1396	0.0394
	Standard error	214.30	127.12	0.0066	0.47	0.0403	0.0114
Nematode-free	Mean	4008.63	2256.75	0.1310	39.68	0.9907	0.2684
	Standard deviation	902.47	404.86	0.0301	1.51	0.2116	0.0570
	Standard error	260.52	116.87	0.0087	0.44	0.0611	0.0165

[a] Average of 12 rice seedlings.

Table 8. Mean[a] of length, number, and dry weight of roots and shoot length, fresh weight and dry weight (six-week old seedlings in growth chamber).

Treatment		Total root length cm	Total no. of roots	Root dry weight g	Shoot length cm	Shoot fresh weight g	Shoot dry weight g
Nematode-infested	Mean	2499.11	1651.42	0.1335	38.18	0.9275	0.2657
	Standard deviation	574.25	370.06	0.0732	2.32	0.2166	0.0633
	Standard error	165.77	106.83	0.0211	0.67	0.0625	0.0183
Nematode-free	Mean	2963.26	1899.00	0.1749	39.98	1.0688	0.3254
	Standard deviation	679.99	363.50	0.0445	2.67	0.2402	0.0844
	Standard error	196.30	104.93	0.0128	0.77	0.0693	0.0244

[a] Average of 12 rice seedlings.

Table 9. Effect of Hirschmanniella oryzae (Van Breda de Haan, 1902) Luc & Goodey, 1963 on rice seedlings growing in growth chamber (statistical significance of the data presented in Tables 6-8).

Seedling age (weeks)	Measurement	Treatment mean difference (nematode-free) - (nematode-infested)	Standard error of mean difference	t value
2	Total root length	858.72	370.49	2.318*
	Total root number	439.09	166.30	2.640*
	Root dry weight	0.0457	0.0098	4.663**
	Shoot length	1.93	0.92	2.098*
	Shoot fresh weight	0.1310	0.0541	2.421*
	Shoot dry weight	0.0356	0.0135	2.637*
4	Total root length	1133.85	337.34	3.361**
	Total root number	362.17	172.68	2.097*
	Root dry weight	0.0126	0.0109	1.156
	Shoot length	2.39	0.64	3.734**
	Shoot fresh weight	0.2021	0.0732	2.761*
	Shoot dry weight	0.0531	0.0201	2.642*
6	Total root length	464.15	256.93	1.807
	Total root number	247.58	149.74	1.653
	Root dry weight	0.0414	0.0247	1.676
	Shoot length	1.80	1.02	1.765
	Shoot fresh weight	0.1413	0.0933	1.515
	Shoot dry weight	0.0597	0.0305	1.957

* Significant (at 5% level of probability).

** Highly significant (at 1% level of probability).

Table 10. Diameters[a] of primary and secondary roots of rice seedlings grown in a growth chamber in nematode-free and nematode-infested soil.

Seedling age (weeks)	Primary roots (microns)			Secondary roots (microns)		
	Nematode-infested	Nematode-free	t value	Nematode-infested	Nematode-free	t value
2	693.97	788.23	3.918**	95.75	106.05	9.196**
4	789.38	813.82	1.168	94.01	103.17	11.171**
6	748.03	793.84	2.144*	90.92	96.57	3.844**

[a] Mean diameters of roots from 12 seedlings.

* Significant (at 5% level of probability).

** Highly significant (at 1% level of probability).

to soil caused reductions in the length of roots, but greater reductions were caused by treatments with nematode suspensions and noninfested rice roots plus the nematode.

Table 12 shows statistical significance of different treatments on rice seedling root numbers. Water from the nematode suspensions or the culture of the microorganisms in that water did not cause significant reduction of root numbers. Noninfested and infested rice roots, added to the soil, caused a reduction in numbers of roots similar to that caused by the water from the nematode suspension and the culture of bacteria and fungi. The nematode and noninfested rice roots plus the nematode caused a statistically significant reduction in the number of roots, and this reduction was greater than that of other treatments.

Statistically significant differences in root dry weight of rice seedlings are shown in Table 13. Noninfested rice roots plus the nematode caused the highest reduction in root dry weight but equivalent reductions were caused by noninfested rice roots, infested roots and H. oryzae added to soil alone. Microorganisms or cultures of those microorganisms from the nematode suspension water did not cause significant reductions in root dry weight.

Table 14 shows statistical significance of differences in the shoot length of rice seedlings. Water from the nematode suspension or the culture of bacteria and fungi from that water did not cause statistically significant reductions in length of shoot. Nematodes or noninfested rice roots, when added to the soil, caused a reduction in shoot length. Infested rice roots or noninfested rice roots plus the nematode caused the highest reductions.

Table 11. Statistical significance of root length of rice seedlings.

Treatment	Mean[a]	Indication of significance[b]
Nematode-free	496.44	a
Culture of bacteria and fungi[c]	471.31	a b
Water from nematode suspension	470.99	a b
Noninfested rice roots added	381.17	b c
Infested rice roots added	320.93	c d
Nematode suspension added	270.18	d
Noninfested rice roots plus nematode suspension	215.75	d

[a] Average of 10 rice seedlings (cm).

[b] Values in the column followed by common letters are not significantly different at the 5% level.

[c] Culture of the bacteria and fungi from water of nematode suspension.

Table 12. Statistical significance of number of roots of rice seedlings.

Treatment	Mean[a]	Indication of significance[b]
Nematode-free	529.60	a
Water from nematode suspension	465.20	a b
Culture of bacteria and fungi[c]	458.10	a b
Noninfested rice roots added	414.60	b
Infested rice roots added	361.70	b
Nematode suspension added	257.10	c
Noninfested rice roots plus nematode suspension	236.80	c

[a] Average of 10 rice seedlings.

[b] Values in the column followed by common letters are not significantly different at the 5% level.

[c] Culture of the bacteria and fungi from water of nematode suspension.

Table 13. Statistical significance of root dry weight of rice seedlings.

Treatment	Mean[a]	Indication of significance[b]
Water from nematode suspension	0.0164	a
Culture of bacteria and fungi[c]	0.0163	a
Nematode-free	0.0153	a
Noninfested rice roots added	0.0138	b
Infested rice roots added	0.0132	b
Nematode suspension added	0.0132	b
Noninfested rice roots plus nematode suspension	0.0088	c

[a] Average of 10 rice seedlings (gram).

[b] Values in the column followed by common letters are not significantly different at the 5% level.

[c] Culture of the bacteria and fungi from water of nematode suspension.

Table 14. Statistical significance of shoot length of rice seedlings.

Treatment	Mean[a]	Indication of significance[b]
Water from nematode suspension	47.73	a
Culture of bacteria and fungi[c]	47.10	a
Nematode-free	46.64	a
Nematode suspension added	43.01	b
Noninfested rice roots added	42.22	b
Noninfested rice roots plus nematode suspension	39.37	c
Infested rice roots added	38.60	c

[a] Average of 10 rice seedlings (cm).

[b] Values in the column followed by common letters are not significantly different at the 5% level.

[c] Culture of the bacteria and fungi from water of nematode suspension.

The results in Table 15 indicate that the water from the nematode suspension or the culture of the bacteria and fungi from that water did not cause reductions in fresh weight of shoots. Noninfested rice roots or the nematode, when added to the soil, caused reductions of shoot fresh weight. Reductions in shoot fresh weight were highest with additions of infested rice roots or noninfested rice roots plus the nematode.

In Table 16, the results show that the water from the nematode suspension or the culture of the microorganisms in that water did not cause statistically significant reductions in dry weight of shoots. However, other treatments caused reductions in shoot dry weight. The nematode and noninfested rice roots, when added to the soil, caused reductions in the dry weight of shoots. The highest reductions in shoot dry weight resulted from additions of infested rice roots and noninfested rice roots plus the nematode.

Total phenolic compounds in rice roots and nematodes

The results on determinations of total phenolic compounds (Fig. 6) indicate that the infestation of nematodes caused an increase in total phenols. At 3 and 7 days after inoculation the amount of total phenolic substances in nematode-infested roots was about 2 times greater than in nematode-free roots; this difference became less at 14 days after inoculation. It was found also that Hirschmanniella oryzae contained trace amounts of phenolic compounds (7.5 micrograms per 1000 nematodes).

Table 15. Statistical significance of fresh weight of shoots of rice seedlings.

Treatment	Mean[a]	Indication of significance[b]
Water from nematode suspension	0.8173	a
Nematode-free	0.7635	a
Culture of bacteria and fungi[c]	0.7493	a
Noninfested rice roots added	0.6284	b
Nematode suspension added	0.6152	b
Infested rice roots added	0.4552	c
Noninfested rice roots plus nematode suspension	0.4499	c

[a] Average of 10 rice seedlings (gram).

[b] Values in the column followed by common letters are not significantly different at the 5% level.

[c] Culture of the bacteria and fungi from water of nematode suspension.

Table 16. Statistical significance of shoot dry weight of rice seedlings.

Treatment	Mean[a]	Indication of significance[b]
Water from nematode suspension	0.1417	a
Nematode-free	0.1372	a b
Culture of bacteria and fungi[c]	0.1359	a b
Nematode suspension added	0.1167	b c
Noninfested rice roots added	0.1063	c
Infested rice roots added	0.0817	d
Noninfested rice roots plus nematode suspension	0.0799	d

[a] Average of 10 rice seedlings (gram).

[b] Values in the column followed by common letters are not significantly different at the 5% level.

[c] Culture of the bacteria and fungi from water of nematode suspension.

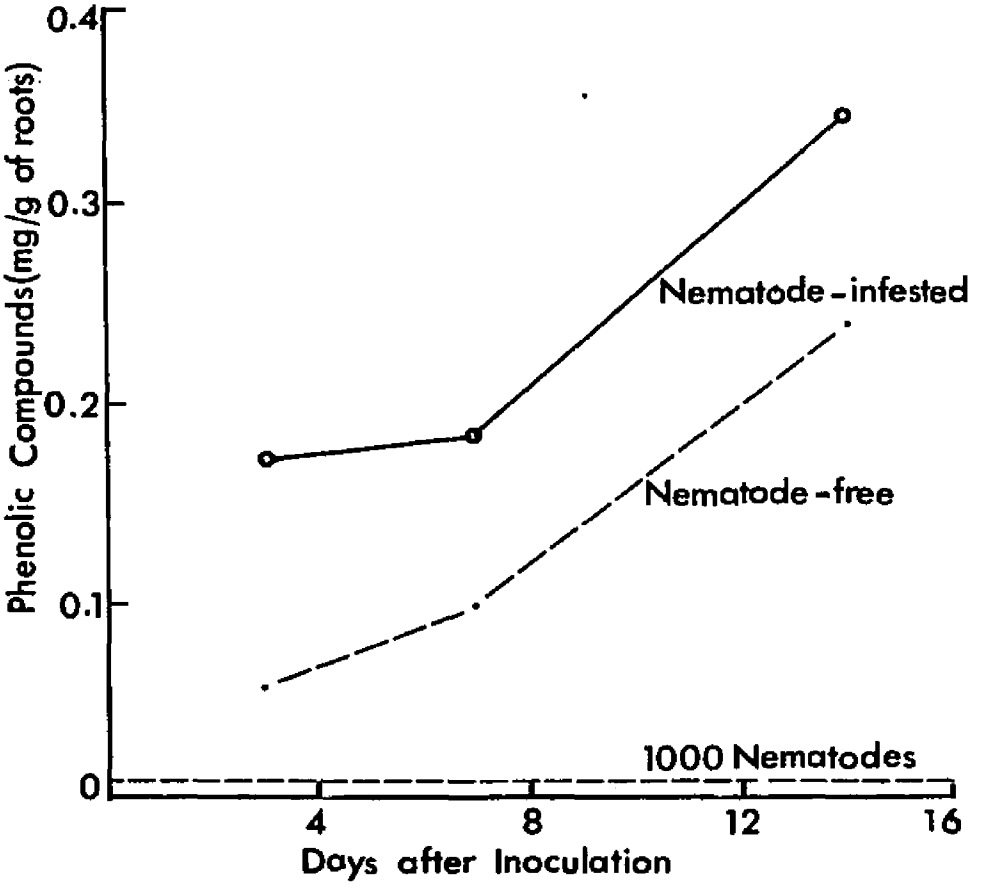

Figure 6. Total phenolic compounds found in nematode-infested and nematode-free rice roots and in 1000 specimens of Hirschmanniella oryzae.

Enzymatic activity

A) Polyphenol oxidase:

Polyphenol oxidase activities in nematode-infested and nematode-free rice roots at 3, 7 and 14 days after inoculation are shown in Fig. 7, 8 and 9, respectively. In all cases, the activity of the enzyme in nematode-infested roots was higher than in nematode-free roots. In another experiment, rice roots were punctured with a sterilized needle and the enzyme activity was determined at 3 and 7 days after root injury. It was found that 3 days after root injury, the polyphenol oxidase activity was greater than in healthy roots, but it was not as high as that in nematode-infested roots (Fig. 10). Seven days after the roots were punctured, the enzyme activity was at the same level as in healthy roots (results not shown).

B) Catalase:

The results (Fig. 11) show that 3 days after inoculation of rice roots with the nematode the catalase activity was about 2 times greater than that in nematode-free roots. At 7 days after inoculation the difference in enzyme activity was less than on the third day. At 10 and 14 days after inoculation there was almost no difference in the activity of the enzyme between the two treatments.

C) Beta-glucosidase:

The activities of beta-glucosidase enzyme in rice roots at 7 days after inoculation and in H. oryzae are shown in Table 17. Activity of the enzyme in nematode-infested roots was almost 2 times greater than that in nematode-free roots. It was found also that

H. oryzae secreted or excreted beta-glucosidase into the nematode suspension water.

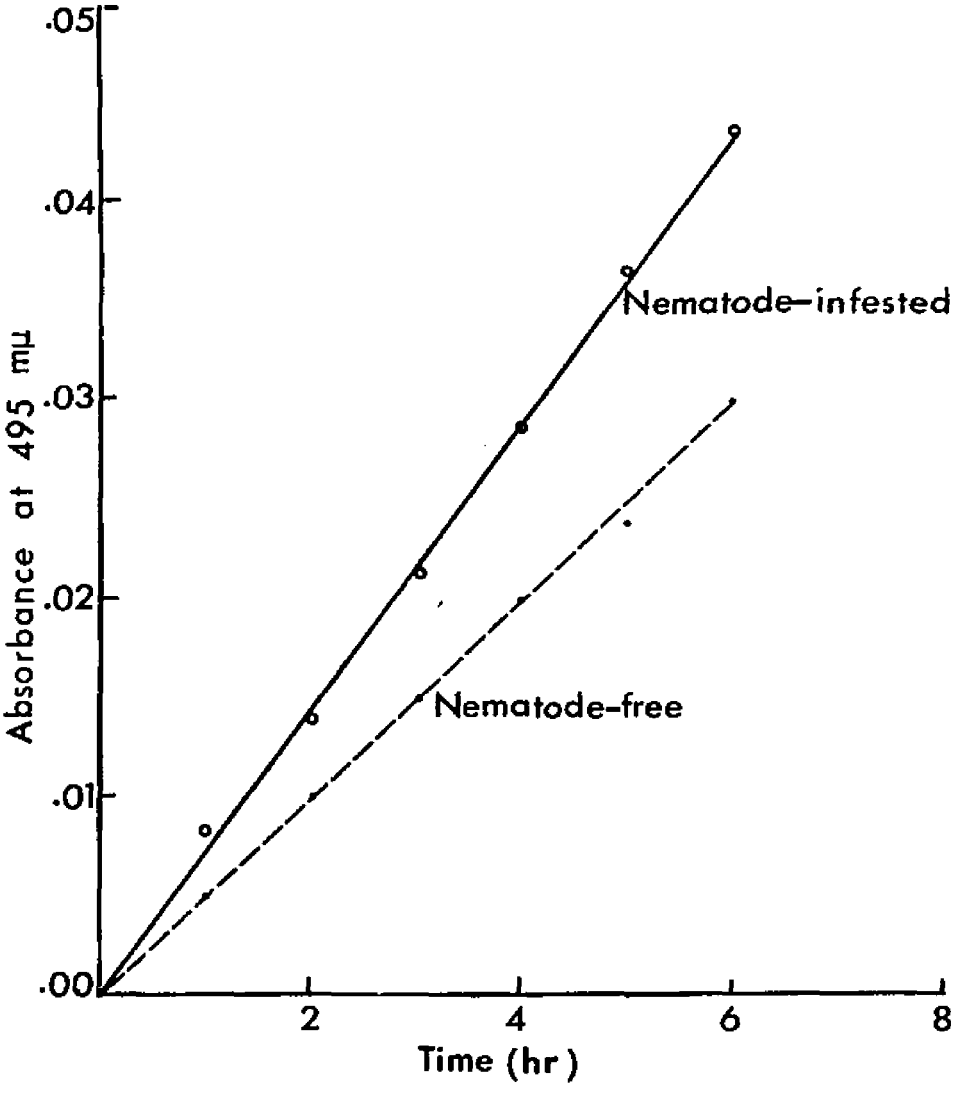

Figure 7. Polyphenol oxidase activity in nematode-infested and nematode-free rice roots at 3 days after inoculation of rice seedlings with Hirschmanniella oryzae.

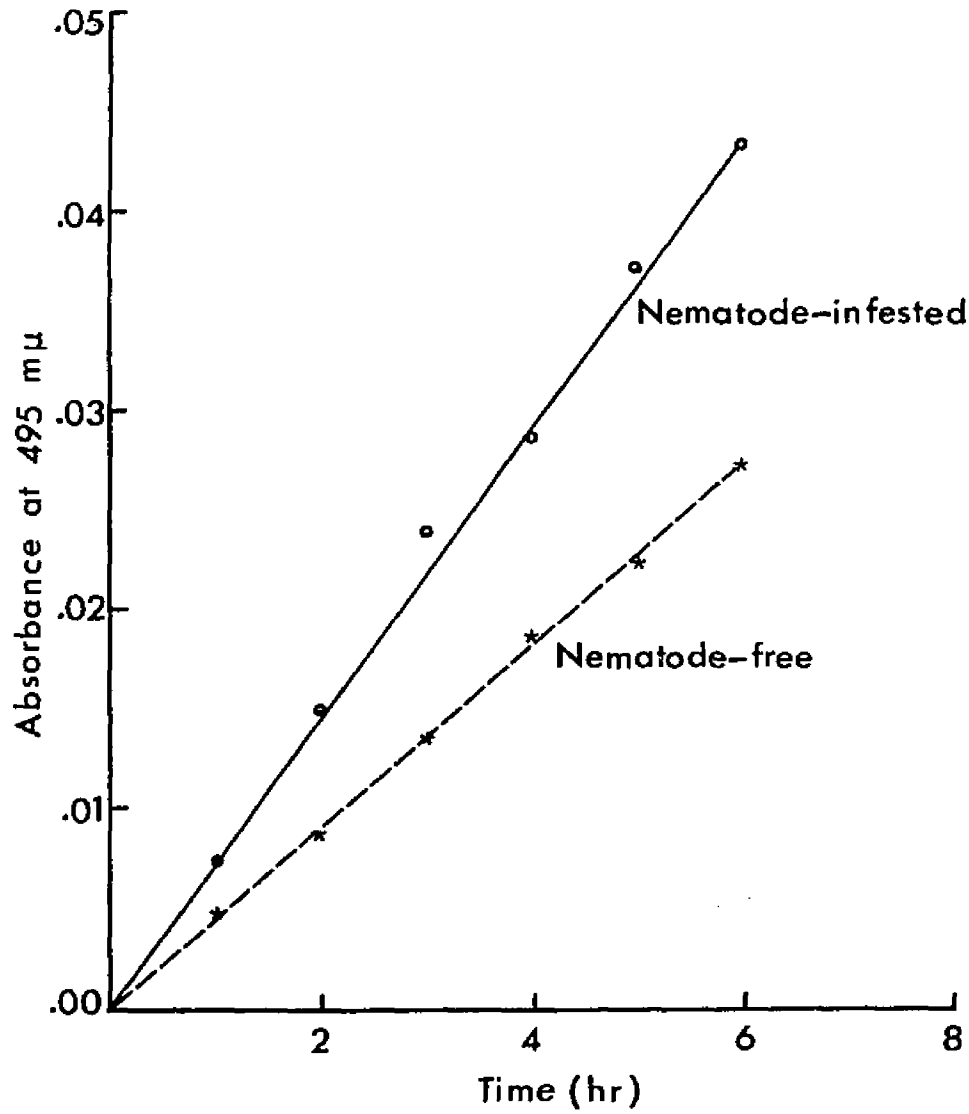

Figure 8. Polyphenol oxidase activity in nematode-infested and nematode-free rice roots at 7 days after inoculation of rice seedlings with Hirschmanniella oryzae.

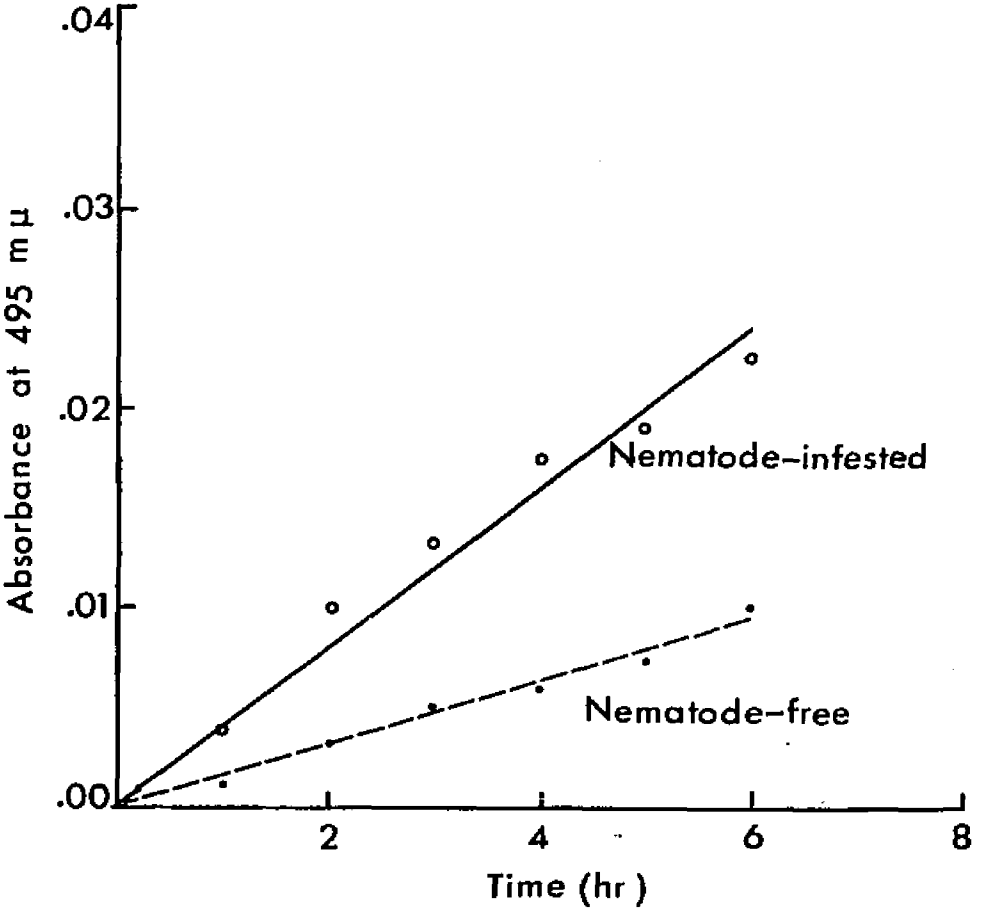

Figure 9. Polyphenol oxidase activity in nematode-infested and nematode-free rice roots at 14 days after inoculation of rice seedlings with Hirschmanniella oryzae.

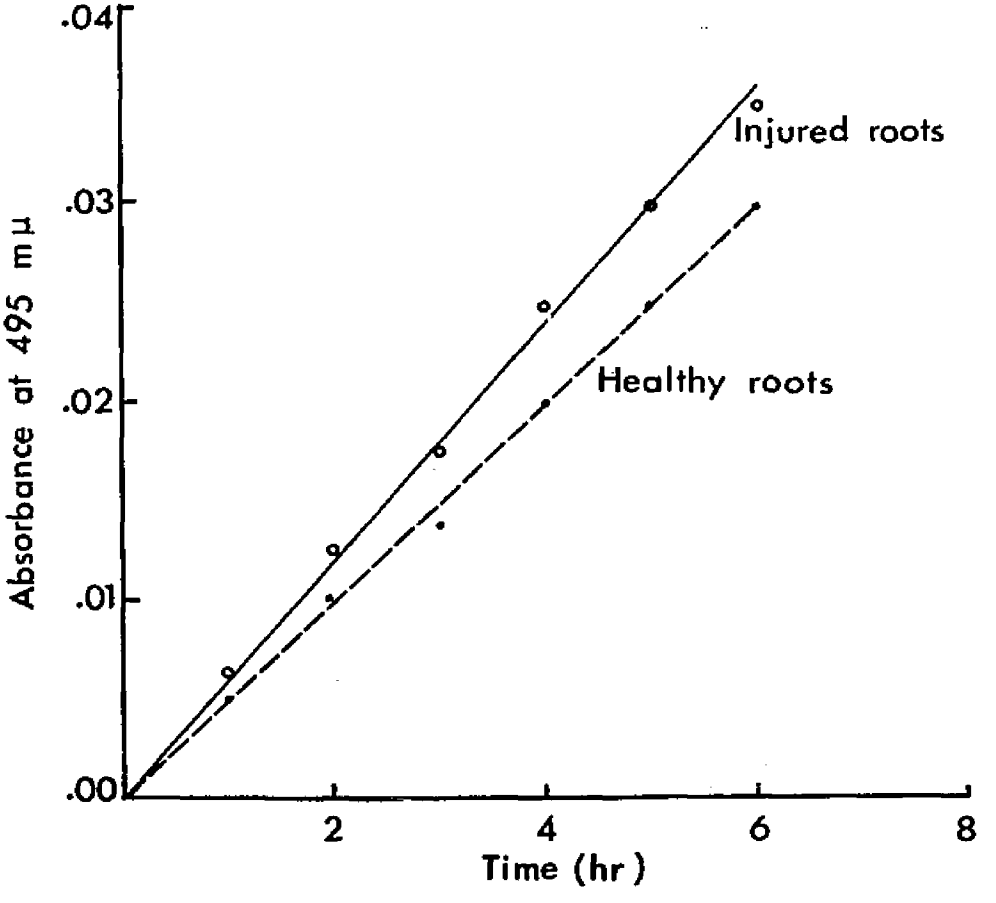

Figure 10. Polyphenol oxidase activity in healthy and injured rice roots 3 days after roots were punctured with a sterilized needle.

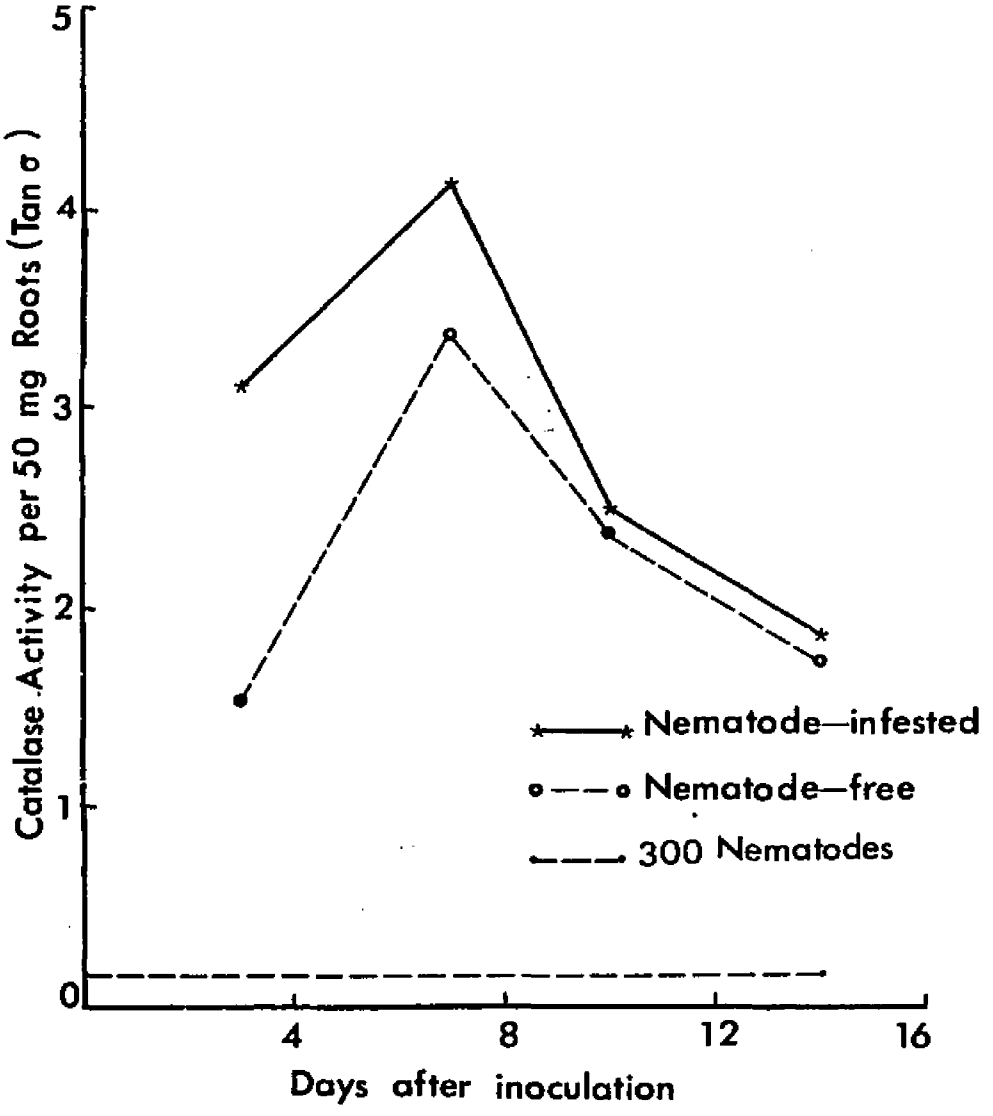

Figure 11. Catalase activity in nematode-infested and nematode-free rice roots and in 300 specimens of Hirschmanniella oryzae.

Table 17. Beta-glucosidase activity of rice root and nematode homogenate extracts and nematode wash water.

Treatment	g p-nitrophenol released per g root fresh weight
Nematode-infested roots[a]	3100
Nematode-free roots	1750
1000 H. oryzae	226
H. oryzae wash water	20

[a] Rice roots extracted 7 days after inoculation with H. oryzae.

DISCUSSION

Observations on the feeding of Hirschmanniella oryzae (Van Breda de Haan 1902) Luc and Goodey 1963 on rice seedling roots revealed that the nematode can destroy parenchyma cells and form rounded large cells or cavities in the cortex of rice roots. The destruction of cell walls and the formation of cavities as a result of nematode movement through roots was reported also in the nematodes, Radopholus similis (7, 11) and Pratylenchus penetrans (68), which are related taxonomically to H. oryzae.

Besides the production of cavities in roots, H. oryzae also causes necrosis of roots. This is in agreement with Van der Vecht (71) who observed that H. oryzae caused discoloration of root tissues, especially adjacent to the stele and near the base of lateral roots. The closely related nematode, Pratylenchus penetrans, is a strongly pathogenic species which causes severe and extensive root necrosis in a wide range of crops (47, 48, 51, 59, 68, 69). Radopholus similis and Helicotylenchus multicinctus can cause necrotic lesions in banana roots (7).

A discussion of nematode-plant dynamics is now possible with reference to numbers of H. oryzae in roots, the age of seedlings, and the severity of stunting effects on the seedlings.

When rice seeds are soaked in water for 12 hours and then retained in a moist condition for 4 days, only one primary seminal root and a few adventitious roots are developed. When these rice seedlings are planted in nematode-infested soil, roots are infested by

the nematode and the reduction in growth is evident, especially in seedlings 2 weeks after planting. In the greenhouse experiment, a few nematodes had invaded the roots at two weeks after planting and this low number is apparently related to a reduction in the stunting effect 4 weeks after inoculation. Later, seedlings produce more roots, at the same time that the number of invading nematodes are increasing rapidly; and this is related to significant reductions in growth which occurred in 6-week-old seedlings.

In the growth chamber experiment, conditions were such that rice seedlings produced more roots than in the greenhouse and at two weeks after inoculation the nematode numbers in roots in growth chamber seedlings were 7 times higher than in the greenhouse seedlings. Because of the initial high infestation of seedling roots by H. oryzae in the growth chamber, a stunting effect was evident at 2 and 4 weeks after inoculation. However, at 4 weeks after inoculation the number of invading nematodes had not increased beyond the number found at 2 weeks; this resulted in some recovery of plant vigor and an absence of any significant reduction in root and shoot measurements.

The effects of H. oryzae on rice seedlings have been reported in various papers. Van der Vecht and Bergman (72) found that the nematode caused a reduction in shoot length and tillering. They mentioned that the relation between the nematode and rice plant is very complex, depending partly on the number of nematodes, time of infestation, physical and chemical soil conditions, rice varieties, agricultural practices and meteorological conditions. Reductions of shoot length, shoot fresh weight and dry weight caused by H. oryzae were reported by Kawashima and Fujinuma (41). Whitlock (75) found the

nematode caused no damage to germinating and young rice seedlings when he inoculated 50 specimens of H. oryzae around 10 rice grains in 4-inch pots.

A report that H. oryzae causes increases in tillering, root length and root weight was made by Rao and Panda (55). The increases in tillering, root length and weight were in accordance with increase of inoculum up to a level of 5 and 10 thousand nematodes per plant, although Rao and Panda found only very small numbers of nematodes in the roots.

Bacteria and fungi from nematode wash water caused no significant reductions in root and shoot measurements. Noninfested rice roots, infested rice roots, nematode suspensions, and noninfested rice roots plus nematode suspensions caused significant reductions in root and shoot measurements. The combination of noninfested rice roots and nematodes caused a greater reduction of rice seedling growth than any of the other soil amendments.

Acharya (1) studied the decomposition of rice straw in soil under flooded conditions. He found that products of the decomposition were acetic acid, butyric acid, carbon dioxide, methane, and negligible amounts of hydrogen. Ponnamperuma (53) pointed out that these reduction products can harm rice by weakening respiration, growth and nutrient uptake of rice plants and by direct poisoning of the plants. These conclusions agree with the results of soil amendment studies in this work. When rice roots were added to soil, subsequently submersed, fermentation occurred and the growth of rice seedlings was inhibited. The addition of nematodes to rice seedlings growing under these conditions produced the highest growth retardation. I interpret

this as a combined effect, on rice seedlings, of nematodes and organic acids produced by decomposition of rice roots, because these levels of organic acids would inhibit seedlings without adversely affecting the nematodes (26, 27, 28). This is in agreement with the observations of Van der Vecht and Bergman (72) that the most serious injury resulting from H. oryzae infestations of rice occurred under conditions which did not enable plants to recover.

In this study it was found that the levels of phenolic compounds increased in rice seedlings infested with H. oryzae. This increase must be attributed to the response of the plant to nematode infestation because only a very small amount of phenolic compounds is present in the nematode.

Accumulation of phenolic compounds is one of the characteristic responses of plant tissue to infestion by fungi (6, 10, 23, 57, 74), viruses (35) and nematodes (48,51,68, 69). Wakimoto and Yoshii (74), working on polyphenols in rice plants, concluded that there were more than 8 polyphenolic compounds present. The four principal compounds appeared to be ortho-dihydric phenols. They found also that the polyphenol content of rice leaves increased gradually with growth of the plant and reached a maximum level at the tillering stage. It has been shown that the polyphenol content of rice leaves increases when leaves are infected with the rice blast fungus (Piricularia oryzae) or with the brown leaf spot fungus (Helminthosporium oryzae). Townshend (68, 69) detected phenolic compounds in roots of celery and strawberry infested with Pratylenchus penetrans. He found that the development of discoloration and necrosis appeared to be correlated, in part, with the presence and the distribution of phenolic compounds

in the roots. A similar relationship has been demonstrated in peach and apple (48, 51).

The presence of phenolic compounds in nematodes has been reported by several workers. Ellenby (12), using histochemical techniques, detected polypenols in the exo- and endocuticle of the wall of cysts of Heterodera rostochiensis. Clarke et al. (8) found that dried eggshells of this nematode contained 3% polyphenols. The cyst wall of H. rostochiensis was found to contain 2% polyphenols (9).

The present study showed that there was an increase in polyphenol oxidase activity in rice seedlings infested with H. oryzae. Increase in the activity of this enzyme was found also in rice roots punctured with a needle. A similar increase in polyphenol oxidase activity due to cellular injury has been reported voluminously elsewhere. Jennings et al. (33) reported an increase in polyphenol oxidase activity in susceptible maize infected with Helminthosporium carbonum. In Rhizoctonia-infected bean hypocotyls, a marked increase in the activity of phenol oxidase was found only in the lesions and during the time when the lesions were turning brown (46). Farkas et al. (13) found an increase in polyphenol oxidase activity in detached leaves and in diseased tissues. They claimed that this increase is not due to de novo enzyme protein synthesis, but appears connected with the breakdown of cellular proteins. Similar results in which tobacco mosaic virus causes an increase in polyphenol oxidase activity in diseased tobacco was reported by Jockusch (34) and Kammen and Brouwer (36). Toyoda and Suzuki (70) observed that polyphenol oxidase activity was absent in homogenates of rice leaves infected with Piricularia oryzae while the activity of peroxidase was enhanced.

They inferred that the brown spots caused by this disease were produced as a result of oxidation of polyphenols by peroxidase.

Barat et al. (5) studied catalase activity in rice roots from seedlings of different ages. They found that the enzyme activity reached the highest level around the panicle formation stage. Immediately after the heading stage the catalase activity declined rapidly and finally disappeared with aging of the plant. They assumed that around the panicle formation stage, catalase plays an important role in conversion from the vegetative to the reproductive condition. Maxwell and Bateman (46), working on Rhizoctonia-infected bean hypocotyls, found that there was a marked increase in the activity of catalase only in the lesions, at the period during which the lesions became brown. This is in agreement with Rudolph and Stahmann (58) who found that the "greasy" infection centers of the leaves in the bacterial disease known as halo blight of bean contained a large amount of catalase enzyme whereas only low catalase activity was found in the surrounding yellow halos and no activity was detected in the surrounding green leaf tissue.

Hussey and Krusberg (29), working on disc-electrophoretic patterns of enzymes and soluble proteins of Ditylenchus dipsaci and D. triformis, found two catalase isoenzymes in homogenates of these nematodes but they were unable to demonstrate whether the nematodes secreted the enzymes.

In this study it was found that the catalase enzyme activity in nematode-infested rice roots was increased, especially at the early stage of infestation. Later, the activity of the enzyme declined to a normal level. At 3 days after inoculation, the catalase activity

in nematode-infested rice roots was about double that of nematode-free roots. At this stage, the lesions were newly developed, contained only a few nematodes and were relatively free of bacterial contamination. These reasons support the view that the increased catalase activity resulted from the response of the host.

It is generally known that almost all low molecular weight phenols occur in living plant cells in combined form, usually as glycosides (24). Invasion of tissues by microorganisms would release glycosidases by breakdown of the plant cells and these enzymes could then liberate phenol from plant glycosides (54). Pathogenic microorganisms, especially facultative parasites, can excrete also considerable amounts of hydrolytic enzymes, including various glycosidases into the surrounding medium (57). In apples leaves, upon injury by Venturia inaequalis, a beta-glucoside (phloridzin) is hydrolysed by a host beta-glucosidase to yield the phenol--phloretin which is oxidized to give the products that inhibit the development of the invading pathogen (50). Davis et al. (10), after a study of conjugated phenols in the Fusarium wilt syndrome, suggested that the pathogen produces hydrolytic enzymes which liberate phenols from beta-glucosides and that the host's phenol oxidases oxidize free phenols to colored products.

Mountain and Patrick (48) proposed the existence of beta-glucosidase in the nematode, Pratylenchus penetrans, although they were not able to demonstrate it. The occurrence of beta-glucosidase enzyme in larvae of Heterodera rostochiensis was detected by Wilski and Giebel (76). The found that the homogenate of a biotype of this nematode which could produce necrosis of potato tubers contained 5

times as much enzyme activity as the biotype that could not produce necrosis. The nematode was found also to secrete the enzyme into the wash water.

In this study H. oryzae produced the enzyme beta-glucosidase which was detected in the wash water. Activity of beta-glucosidase in nematode-infested rice seedling roots was almost double that of nematode-free seedling roots. This increase in enzyme activity may result from secretion by nematodes and by host cells damaged by the nematode.

SUMMARY

1. Feeding of Hirschmanniella oryzae (Van Breda de Haan 1902) Luc and Goodey 1963 was observed in the laboratory. The nematode fed intracellularly on the primary root in the region of emergence of secondary roots and in the root hair region but not at the root tip. Feeding caused the disruption of cell walls, formation of cavities and necrosis of roots.
2. The infestation by H. oryzae of rice root seedlings growing in the greenhouse caused significant or highly significant reductions in total root length, root dry weight, shoot length, shoot fresh weight and dry weight in 2-week-old seedlings. The retardation of growth of rice seedlings declined in 4- and 6-week-old seedlings.
3. In growth chamber tests, the nematode caused significant or highly significant reductions in root and shoot measurements at 2 and 4 weeks after inoculation. Then the plants tended to recover and the retardation of growth disappeared in 6-week-old seedlings.
4. Bacteria and fungi from nematode wash or suspension water did not cause significant reductions in growth of rice seedlings.
5. Rice seedlings grown in soil amended with noninfested or infested rice roots showed an inhibition in growth which became more severe when specimens of H. oryzae were added to rice seedlings growing under these conditions.
6. Infestation of rice seedling roots by H. oryzae caused an increase in phenolic compounds in the roots. The nematode itself contained a trace amount of total phenols (7.5 micrograms per 1000 nematodes).

7. Polyphenol oxidase activity in nematode-infested rice roots was higher than in nematode-free roots. A slight increase in the activity of this enzyme was found also in roots 3 days after mechanical injury was made.
8. Catalase activity increased in the roots only at an early stage of nematode infestation. The activity of this enzyme declined to a normal level in roots at about 10 days after inoculation of seedlings. The enzyme was found also in nematode homogenate.
9. The activity of beta-glucosidase enzyme increased in nematode-infested rice seedling roots. The nematode itself contained this enzyme and secreted the enzyme into nematode suspension water. Increase in the activity of this enzyme in the host came from both the host response to nematode infestation and from the nematode secretion of the enzyme.

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VITA

Sman Keoboonrueng was born in Chachoengsao, Thailand on January 10, 1936. He attended elementary school in Chachoengsao and was graduated from a secondary school in Bangkok in March 1956. In the same year he enrolled in the Faculty of Science, Chulalongkorn University where he was graduated with a B.S. in Botany in June, 1962. He worked as a technician in the Narcotic Hospital, Ministry of Public Health from June until December 1962. In January 1963 he moved to the Technical Division, Rice Department, Ministry of Agriculture. He was awarded a study leave from the same department to work toward the M.S. and Ph.D. degrees in the U.S.A. in August 1966. He enrolled in Louisiana State University in September 1966 and received his M.S. degree in Plant Pathology in August 1968. He is a candidate for the degree of Doctor of Philosophy in Plant Pathology in August 1971.

EXAMINATION AND THESIS REPORT

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Major Field: Plant Pathology

Title of Thesis: Effects of Rice-root Nematode, Hirschmanniella oryzae (Van Breda de Haan 1902) Luc and Goodey 1963 on Rice Seedlings

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